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SEED TREATMENT AND RAINFALL IN RELATION TO THE CONTROL OF CABBAGE BLACK-LEG.

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INTRODUCTION.

The black-leg of cabbage, first noted as occurring in America by Manns (*7*),[2] has become one of the most serious and widespread maladies of this crop. Henderson (*3*), who, on the basis of his earlier studies in Wisconsin, has given the most complete treatise on the disease, demonstrated that cabbage seeds which had been invaded by the causal fungus, *Phoma lingam* (Tode) Desm., were a common

[1] The major portion of the work reported upon herewith was carried on at the laboratory of plant pathology of the University of Wisconsin as a cooperative project between the university and the United States Department of Agriculture. Along with this, the writer has had opportunity for field studies in some of the chief cabbage-growing sections in the Eastern, Western, and Southern States. The writer wishes to express his appreciation to Prof. L. R. Jones for the helpful advice and criticisms received during the course of the investigation and to Dr. W. B. Tisdale and Miss Ruth Tillotson for assistance in part of the field observations and laboratory experiments.

[2] The serial numbers (italic) in parentheses refer to "Literature cited" at the end of this bulletin.

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source of primary inoculum the following year. As he pointed out, it has been the experience of many growers that the appearance of this disease in epiphytotic form in a given locality is usually associated with a certain lot of cabbage seed. Observations by the writer have strengthened the belief that infected seed is the chief cause of heavy losses from black-leg.

Henderson (*3*) found that naked pycnospores of *Phoma lingam* were killed within two or three minutes by 1:200 formaldehyde or 1:1,000 mercuric-chlorid solutions. Spores within pycnidia embedded in soft cortical tissues of the host were killed after 21 minutes by the formaldehyde solution, while with the other fungicide a few spores were still viable after this length of treatment. Henderson's study was limited by the small number of infected seeds available. It was found, however, that after treatment in 1:200 formaldehyde solution for 21 minutes or in 1:1,000 mercuric-chlorid solution for 10 minutes, infected seeds still yielded pure cultures of *Phoma lingam*.

In 1917 a crop of the yellows-resistant Wisconsin Hollander seed was grown at Racine, Wis. A scattering of *Phoma lingam* developed on the seed plants, and a very small percentage of the pods became infected. All of this seed (approximately 25 pounds) was treated with a 1:256 solution of formaldehyde, and most of it was planted in the same locality the following season. Black-leg developed in epiphytotic form in most of the seed beds, resulting in heavy crop losses where such plants were set in the field. A typical field from this lot of seed as it appeared at the end of the season is shown in Plate I, *A*. The uniformity with which the disease developed in beds from the lot of seed in question and the fact that many of these beds were on soil which had not grown cabbage for many years left no doubt that the fungus carried over in the seed was the source of infection. Moreover, it was evident that despite seed treatment the parasite had spread from comparatively few centers to a large percentage of the plants during their growth in the seed bed.

This situation raised the important question as to how much reliance is to be placed upon seed treatment as a means of control for black-leg. Experiments and observations reported later in this paper show that a very small percentage of infected seeds may under certain environmental conditions cause almost a total loss of the crop. Moreover, the detection of such infected seeds is very difficult, even by an expert. Since a very large portion of the cabbage seed used in this country is grown in a few restricted areas in America and abroad, the average grower uses seed concerning the history of which he knows very little, and this fact makes it the more necessary either that seed treatment be entirely effective or that the production of Phoma-free seed be insured.

It was upon the basis of these facts that the present investigation was begun. It was necessary, first of all, to determine more definitely the limitations of seed treatment with the common disinfectants, both upon commercial cabbage seed and upon the black-leg fungus within infected seeds. Following this, the relation of certain environmental factors to the development and spread of the disease, especially in the seed bed, and their influence upon the ultimate success of seed treatment were studied. This paper is a progress report upon the work along these lines.

EFFECT OF FUNGICIDAL TREATMENT IN THE LABORATORY UPON SEED AND THE SEED-BORNE FUNGUS.

Because of its practical bearing upon the efficacy of seed treatment, an extensive study was made of the resistance of the fungus within infected seeds to heat, desiccation, and chemical fungicides, and, as a necessary corollary, experiments on the effect of the same treatments upon normal cabbage seeds were performed. Early experiments showed that it would be impossible completely to disinfect seed without producing some detrimental effect upon its vitality. From the standpoint of practical cabbage culture, however, seed treatment can be carried somewhat beyond the point of first injury with profit if sufficient benefit is to be derived from fungicidal action. Moreover, as will be pointed out later, where it is desirable to rid small quantities of "mother seed" of the black-leg fungus before introducing the seed into a new locality, quite severe treatment may be desirable with the aim of complete eradication of the parasitic organism. For these reasons, in the laboratory experiments treatment with disinfectants was usually prolonged to the point where complete eradication was attained, and the effect upon commercial seeds up to that point was determined.

Four types of treatment were used: (1) Mercuric chlorid, (2) formaldehyde, (3) hot water, and (4) desiccation at moderate and high temperatures. For the study of the effect of treatment upon the fungus, seeds directly beneath or very close to pod lesions were collected shortly before maturity. Two such lots were used, one (No. 4–18) secured in a field of the Wisconsin Hollander variety at Racine, Wis., in July and August, 1918, and the second (No. 1–19) collected by Mr. E. E. Clayton from a field (variety not determined) at Mattituck, Long Island, N. Y., in July, 1919. The earlier and more extensive experiments were performed with the former lot, and the stronger treatments were repeated or performed for the first time with the latter.

The seeds were exposed to the various treatments and placed on potato-agar plates. They were kept at room temperature for two to four weeks, within which time the fungus, if viable, was identified by its growth on the medium.

Histological studies to trace the course of the mycelium within and beneath the seed coat have not been made. It became evident, however, as a result of cultural studies, that the effect on diseased seeds may vary from little or no change in normal color and little shriveling to very marked shrinkage and entire loss of vitality, dependent upon the extent of invasion by the fungus. That the infection may be confined to the seed coat is shown by one instance where a seed which had been treated with 1:1,000 mercuric chlorid to kill organisms on the surface was placed on a potato-agar plate. Normal germination occurred and the hypocotyl as it developed became attached to the agar about half an inch away from the seed coat. As growth proceeded the young seedling was drawn entirely away from contact with the testa and was later removed to a tube of agar, where it remained sterile. Subsequently, however, a pure culture of *Phoma lingam* developed from the seed coat, proving that the fungus was present in this portion of the seed without having invaded the cotyledons.

In the study of the effect of fungicides upon germination and subsequent development of seedlings from normal cabbage seed, most of the work was done with two lots (Nos. 2–18 and 3–18) of the Wisconsin Hollander variety grown at Racine, Wis., in 1918. In general, one lot (No. 2–18) showed more injury from disinfectants than the other, in spite of the fact that a high percentage of the untreated seeds germinated and produced vigorous seedlings. This may have been due to improper curing, since the seed plants after being harvested were piled indoors before the seed was thrashed and the circulation of air provided was insufficient for rapid and thorough curing. A portion of lot No. 2–18 was stored in a cloth sack in the laboratory, and certain of the treatments were repeated in the second year (winter of 1919–20). After the general limits of injury with these two lots of seed had been determined, the effect of stronger treatments was usually extended to several other lots of various ages and from different sources.

In a few of the early experiments germination tests were made in Petri dishes lined with moistened filter paper. It was found that numerous treated seeds often gave evidence of germination by throwing off the testa and starting growth of the hypocotyl, but development ceased at this point. When parallel tests were made in soil in the greenhouse, most of such seedlings never appeared above ground. Consequently, in order to secure a more definite index of the capacity of various lots of seed for producing vigorous seedlings after treatment, the Petri dish method was discarded. All results reported on the effect of fungicides upon commercial seeds are from tests made in silt-loam soil in the greenhouse. The final estimate of the amount of injury was supplemented in the case of the stronger treatments

by seed-bed trials on silt-loam garden soil at Madison and on sandy soil at Racine, Wis.

In rinsing seed after treatment with mercuric-chlorid or formaldehyde solution one thorough washing in clean tap water was made. As pointed out by Robinson (*9*), who showed that a slight amount of the former fungicide was present in the fourth wash water after treatment of pea, bean, vetch, and barley seeds, it is probable that this one rinsing was insufficient completely to remove the fungicides.

HEAT AND DESICCATION.

Atanasoff and Johnson (*1*) have given a summary of previous work on the relation of high temperatures to the viability of various seeds. They studied further the effect of dry heat upon barley, wheat, rye, and oat seeds and upon a number of seed-borne organisms. It was concluded that the bacteria causing blights of barley (*Bacterium translucens*) and of oats (*Pseudomonas avenae*) could be successfully eradicated from infected seeds by dry heat, while several fungous parasites of cereals were either eliminated or markedly reduced by the same treatment. Harrington and Crocker (*2*) found that the percentage of germination of wheat, barley, Sudan grass, Kentucky bluegrass, and Johnson grass was not materially changed when the moisture content was reduced to less than 1 per cent by drying over calcium oxid or sulphuric acid at room temperature. Waggoner (*10*), by drying radish seeds first at 60° C. and then at 100° C., reduced the moisture content from 4 per cent at normal air-dry condition to 0.4 per cent without reduction of germination.

In the experiments reported here several lots of cabbage seed, varying in age from a few months to 7 years, were reduced to constant weight at 100° C. In these lots the moisture content of the air-dry seeds, untreated, remained around 6 or 7 per cent of the dry weight. The results given in Table I show that at 85° C. the moisture content dropped rapidly during the first 6 hours and then became nearly constant after 48 hours. Seed treated with mercuric-chlorid solution became adjusted to its original air-dry moisture content within a few weeks.

All of the drying experiments reported were carried out in a Freas constant-temperature electric oven. Seeds were placed for this purpose in thin layers in small paper containers, so as to avoid direct contact with substances of high specific heat.

Desiccation at high temperatures (85° to 95° C.) caused a gradual reduction in viability, which was accompanied by retardation in germination and growth. The plants might outgrow the effects of mild treatments, but severe injury was usually permanent. In the early experiments, in which No. 3–18 seed was used, only slight in-

jury to the seed was caused at 96 hours; therefore this method gave some promise as a practical means of control. When it was extended to other seed lots, however, the deleterious effects of the treatment became evident at a much shorter exposure. This was most noticeable in the case of No. 2–18 seed when treated in the second year.

TABLE I.—*Effect of desiccation and heat upon commercial cabbage seeds and upon the black-leg fungus within infected seeds.*

Treatment and length of exposure.	Effect upon infected seeds.				Effect upon commercial seeds.													
					One-year-old seed.										Two-year-old seed.			
					Lot 3–18.[a]			Lot 4–19.[b]			Lot 10–18.[c]		Lot 2–18.[a]		Lot 2–18.[a]		
	Seed lot.	Number of seeds used.	Seeds with viable fungus after treatment (per cent).	Seeds with viable fungus and germinating (per cent).	Dry-weight moisture content (per cent).	Germination (per cent).	Seedling injury.[d]	Dry-weight moisture content (per cent).	Germination (per cent).	Seedling injury.[d]	Germination (per cent).	Seedling injury.[d]	Germination (per cent).	Seedling injury.[d]	Dry-weight moisture content (per cent).	Germination (per cent).	Seedling injury.[d]
Untreated:																	
No exposure	4–18	130	75.3	22.3	....	....	....	....	....	....	....	....	....	....	....	....	....
	1–19	24	66.6	0	6.38	97	○	6.9	92	○	93	○	92	○	7.11	77	○
Exposed at constant temperature:																	
At 85° C.—																	
1 hour	....	....	....	....	....	....	....	3.17	77	⊖	....	....	....	....	2.69	37	+
2 hours	....	....	....	....	....	....	....	2.97	64	⊖	....	....	....	....	2.31	38	+
4 hours	....	....	....	....	....	....	....	1.69	79	⊖	....	....	....	....	2.25	37	+
6 hours	4–18	22	40.9	0	1.27	95	○	1.51	62	÷	....	....	94	○	2.12	33	+
12 hours	4–18	49	32.6	6.1	1.27	97	○	1.42	67	÷	87	○	88	○	1.85	29	+
24 hours	4–18	45	17.7	8.8	1.02	95	○	1.22	54	+	88	○	85	○	1.85	20	+
30 hours	4–18	49	20.4	0	.85	95	○	....	....	....	....	....	90	○	....	....	....
36 hours	4–18	46	4.3	0	.85	96	○	1.29	63	+	....	....	84	○	1.92	32	+
48 hours	4–18	25	36.0	16.0	.72	95	⊖	1.15	42	+	71	÷	74	÷	1.63	22	+
72 hours	4–18	44	9.0	2.2	.93	96	⊖	.73	31	+	58	÷	55	÷	1.32	13	+
96 hours	4–18	49	0	0	.78	87	⊖	1.22	25	+	17	+	53	÷	1.63	14	+
120 hours	....	....	....	....	....	....	....	1.10	17	+	....	....	....	....	1.60	12	+
At 90° C.—																	
12 hours	4–18	24	25.0	0	....	93	○	....	....	....	70	○	78	÷	....	....	....
24 hours	4–18	25	4.0	0	....	63	⊖	....	....	....	77	○	29	÷	....	....	....
36 hours	4–18	24	4.1	0	....	57	÷	....	....	....	70	○	30	+	....	....	....
48 hours	4–18	48	4.1	0	....	10	+	....	....	....	16	÷	0	....	....	....	....
60 hours	4–18	24	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....
72 hours	4–18	47	0	0	....	3	+	....	....	....	38	÷	3	+	....	....	....
At 95° C.—																	
1 hour	....	....	....	....	2.06	86	○	....	46	+	....	....	....	....	2.53	10	++
2 hours	....	....	....	....	1.40	80	○	....	58	+	....	....	....	....	2.09	1	++
4 hours	....	....	....	....	1.02	85	○	....	55	+	....	....	....	....	1.85	5	++
6 hours	....	....	....	....	.68	88	○	....	56	+	....	....	....	....	1.74	2	++
12 hours	4–18	35	2.8	0	.72	87	○	....	53	+	34	÷	66	÷	1.85	1	++
18 hours	4–18	23	0	0	....	....	....	....	....	....	....	....	33	+	....	....	....
24 hours	4–18	24	0	0	.48	77	⊖	....	21	+	8	+	9	+	1.76	2	++
36 hours	4–18	20	0	0	.36	21	÷	....	35	+	2	+	0	....	1.75	1	++
48 hours	....	....	....	....	.25	0	....	....	15	+	....	....	....	....	1.63	0	++
72 hours	....	....	....	....	.31	0	....	....	0	+	....	....	....	....	1.74	0	++
96 hours	....	....	....	....	.27	0	....	....	....	....	....	....	....	....	....	....	....
Dried at 37° to 50° C. 5 days, at 73° 3 days, then at 100° C.:																	
0 hour	....	....	....	....	1.31	90	○	....	....	....	....	....	....	....	....	....	....
12 hours	....	....	....	....	....	22	+	....	....	....	....	....	....	....	....	....	....
24 hours	....	....	....	....	....	0	....	....	....	....	....	....	....	....	....	....	....
36 hours	....	....	....	....	....	0	....	....	....	....	....	....	....	....	....	....	....

[a] Wisconsin Hollander variety grown at Racine, Wis., in 1918.
[b] All Seasons variety grown at La Conner, Wash., in 1919.
[c] Wisconsin All Seasons variety grown at Racine, Wis., in 1918.
[d] Symbols used: ○= No injury; ⊖= slight; ÷= medium; += severe; ++= very severe.

It will not be necessary to consider in detail the data obtained when the seeds were exposed at 90° and 95° C., as listed in Table I. Lot No. 3–18 again proved to be the most resistant, while the remaining lots, as a rule, received marked injury at these temperatures. Parallel treatments of highly infected seeds showed that the fungus had been killed within 18 hours at 95° C. and within 96 hours at 85° C. Both of these treatments might, therefore, be of practical use with lot No. 3–18, but would be very injurious to the other lots.

A quantity of No. 3–18 seed was dried first at 37° to 50° C. for five days, then at 73° C. for three days, by which means the moisture content was reduced to 1.31 per cent. The seed was then placed in an incubator running at 100° C. The results (Table I) showed that in spite of the reduced water content the ability to resist this high temperature was not increased. Agar-plate tests with badly infected seed similarly treated were not made. However, a sample dried at 40° to 50° C. for 19 days and then at 85° C. for one day, the moisture content being reduced to 1.44 per cent of the dry weight, yielded almost as much disease as untreated seed in seed-bed trials. This fact indicates that prolonged drying at moderate temperatures does not materially affect the fungus.

It is evident from the foregoing data that cabbage seed is not uniformly resistant to desiccation at high temperatures. Moreover, the fungus within or beneath the testa is so resistant to this treatment that complete eradication of it from infected seed by baking is impossible without considerable injury to the seed. These facts, together with the difficulty of applying this treatment on a large scale, give doubtful value to this method for commercial use with cabbage seed.

FORMALDEHYDE SOLUTION.

In the trials with formaldehyde solution use was made of formaldehyde, standard strength of approximately 40 per cent, diluted with distilled water. Aside from one treatment with a 1:256 solution for 37 minutes, which was approximately the same as the formula used on a commercial scale in 1918, the stronger concentration (1:128) was used throughout the experiments with formaldehyde. Unless otherwise noted the solutions were used at room temperature, approximately 20° C. Certain of the lots were rinsed in clean water; others were not rinsed. All were spread out in thin layers after treatment and allowed to dry under laboratory conditions. As will be seen from the results given in Table II, the latter treatment always produced greater injury than resulted in the case of corresponding rinsed lots.

The deleterious effect of formaldehyde upon cabbage seed becomes evident both as reduction in percentage of seed germination and as injury to the seedlings. The data in Table II show a progressive decline in the percentage of germination of commercial seeds with the

increase in length of formaldehyde treatment. Injury to the seedlings is evident first from delayed germination and later from various degrees of retardation of growth. A slight check at the outset may be overcome within a few days, but increase in the severity of treatment results in permanent stunting. Accompanying the latter there is usually definite injury to the cotyledons, which remain dwarfed and become distorted in shape. Oftentimes with milder treatments this injury is confined to that cotyledon adjacent to the seed coat, while the other develops normally.

TABLE II.—*Effect of formaldehyde solution upon the black-leg fungus within infected cabbage seeds and upon commercial seeds.*

Strength and temperature of solution.	Length of time in the solution (minutes).	Subsequent treatment.	Effect upon infected seeds.				Effect upon commercial seeds.		
			Seed lot.	Number of seeds used.	Seeds with viable fungus after treatment (per cent).	Seeds with viable fungus and germinating (per cent).	Germination (per cent).		Seedling injury.[b]
							Seed lot 2-18.[a]	Seed lot 3-18.[a]	
Untreated			4-18	130	75.3	22.3	93	97	○
Strength 1 to 256			1-19	24	66.6	0	.....	.....	.....
At room temperature	30	Rinsed	1-19	10	50.0	10.0	.....	.....	.....
Do	37	do	4-18	24					
Strength 1 to 128:					33.3	0	92	98	○
At room temperature	15	do	4-18	25	16.0	0	90	.....	○
Do	30	do	4-18	25	16.0	8.0	85	96	⊖
Do	30	do	1-19	15	60.0	13.3	.....	.....	.....
Do	30	Unrinsed	1-19	23	13.0	0	66	80	+
Do	45	Rinsed	4-18	25	24.0	4.0	79	85	÷
Do	60	do	4-18	25	16.0	4.0	78	88	+
Do	60	Unrinsed	4-18	25	0	0	57	69	++
Do	120	Rinsed	1-19	24	8.3	0	48	.....	++
Do	120	Unrinsed	4-18	25	0	0	46	.....	++
At 55°C	1	do	.....	.....	.....	.....	45	.....	++
Do	2	do	.....	.....	.....	.....	46	.....	++
Do	5	do	.....	.....	.....	.....	28	.....	++
At 60°C	1	do	.....	.....	.....	.....	32	.....	++
Do	2	do	.....	.....	.....	.....	22	.....	++
Do	5	do	.....	.....	.....	.....	20	.....	++

[a] Wisconsin Hollander variety grown at Racine, Wis., in 1918.
[b] Symbols used: ○=No injury; ⊖=slight; ÷=medium; +=severe; ++=very severe.

Table II shows that severe injury with 1 : 128 formaldehyde solution resulted with one hour of treatment when the seed was subsequently rinsed and with 30 minutes when the seed was dried without rinsing. It is also shown that, although materially reduced, the fungus had not been completely eradicated from infected seeds by either of these treatments. Complete disinfection was not attained with rinsed seed even after two hours of treatment and only after one hour with unrinsed seed. It is worthy of note that the infected seeds were killed somewhat earlier than the invading fungus, and this in practice might reduce somewhat the number of primary infections. It will be shown later, however, that infected seeds although incapable of germination

may serve as sources of subterranean infection of near-by seedlings. Treatments with solutions heated to 55° and 60° C. for the very short periods of 1 to 5 minutes were so injurious that their effect upon infected seeds was not given further consideration. It was thus very evident that complete disinfection of infected seeds with a formaldehyde solution is impossible without material injury to the subsequent development of the plants. Moreover, the fact that protracted treatment with this fungicide results in very serious injury to the host plant precludes its use even in those special cases where complete eradication of the parasite is so much desired that moderate seedling injury would be tolerated.

HOT WATER.

The use of hot-water treatment for the control of cabbage black-leg was first suggested by Norton (*8*). He treated cabbage seed for 5 minutes at 60°, 10 minutes at 56°, 15 minutes at 56°, and 20 minutes at 54° C. without injuring it, while spores of the fungus were killed at somewhat lower temperatures. The effect of these treatments upon the fungus within infected seeds was not determined, however.

TABLE III.—*Effect of hot-water treatment upon the black-leg fungus within infected cabbage seeds and upon commercial seeds.*

Temperature.	Length of exposure (minutes).	Effect upon infected seeds.				Effect upon commercial seeds.					
						One-year-old seed.				Two-year-old seed.	
						Lot 2-18.[a]		Lot 4-19.[b]		Lot 2-18.[a]	
		Seed lot.	Number of seeds used.	Seeds with viable fungus after treatment (per cent).	Seeds with viable fungus and germinating (per cent).	Germination (per cent).	Seedling injury.[c]	Germination (per cent).	Seedling injury.[c]	Germination (per cent).	Seedling injury.[c]
Untreated	.....	4-18	130	75.3	22.3	93	○	97	○	67	○
Do	.....	1-19	24	66.6	0	.....	.....	.....	.....	.....	.....
At 50° C.	30	1-19	15	0	0	86	⊖	91	○	66	⊖
Do	60	1-19	20	0	0	85	⊖	82	○	46	÷
At 55° C.	5	1-19	24	29.1	0	81	⊖	86	○	51	÷
Do	10	1-19	35	14.2	0	80	⊖	86	⊖	48	+
Do	15	4-18	17	0	0	67	÷	82	⊖	33	+
Do	15	1-19	35	2.8	0	67	÷	.....	.....	.....	.....
At 56 to 57° C.	20	4-18	23	0	0	.....	.....	.....	.....	.....	.....
At 55° C.	30	1-19	24	0	0	52	÷	39	+	18	+
At 60° C.	5	.....	.....	.....	.....	59	÷	.....	.....	.....	.....

[a] Wisconsin Hollander-variety grown at Racine, Wis., in 1918.
[b] All Seasons variety grown at La Conner, Wash., in 1919.
[c] Symbols used: ○=No injury; ⊖=slight; ÷= medium; +=severe.

The effect of hot water held at 50°, 55°, and 60° C. both upon commercial and infected seeds has been determined by the writer, and the results of the tests are given in Table III. Treatment for 30 minutes at 55° C. completely eradicated the fungus, but considerable

injury resulted to commercial seed held at this temperature for as short a time as 5 minutes. At 50° C. the fungus was killed in 30 minutes with the small number of seeds used, but here, again, some detrimental effect upon commercial seed occurred; in fact, seed injury was evident in somewhat milder treatments than those reported by Norton (*8*). This is probably due to differences in the susceptibility of individual seed lots. Soaking the seed in water before treatment did not appear to be of any distinct advantage. At present, hot-water treatment does not appear to be sufficiently superior to the mercuric-chlorid treatment described later to warrant its recommendation for general use. Further comparative trials, however, should be made.

MERCURIC-CHLORID SOLUTION.

Solutions of mercuric chlorid were made by dissolving the chemically pure salt in distilled water. The treatments were all conducted at room temperature, about 20° C. Four methods were used: (1) Soaking the cabbage seed in the disinfectant without previous treatment and rinsing once in clean water after removal; (2) soaking in the disinfectant without previous treatment and allowing the seed to dry without subsequent rinsing; (3) soaking in water for one to four hours before placing in the disinfectant and rinsing once in clean water after treatment; (4) dipping the seed first in alcohol for two minutes, then transferring to the disinfectant for the desired time, and finally rinsing in clean water. Mercuric chlorid caused a gradual reduction in the percentage of germination of seeds proportionate to the length of soaking and the concentration of the solution. Injury to the germinating seedlings was characterized by temporary or permanent stunting of growth and varied amounts of necrotic tissue extending back from the margins of the cotyledons. The results of experiments with mercuric-chlorid solutions are presented in Table IV.

With the first method (soaking in the disinfectant and rinsing in water) little injury resulted from a 30-minute treatment with the 1:500 solution, and practically none from less severe treatments. In the case of one lot of imported cabbage seed of rather low vitality (not cited in Table IV), considerable injury resulted from the 30-minute 1:500 treatment, indicating that treatments as severe as this should be used with caution. In the case of most lots of cabbage seed, however, the soaking may be prolonged to one or two hours with only slight injury, and at three hours only medium retardation may be expected. These stronger treatments completely eradicated the fungus in the case of the No. 4–18 seed. The fungus in the No. 1–19 lot appeared to be much more resistant to this chemical.

TABLE IV.—*Effect of mercuric-chlorid solution upon the black-leg fungus within infected cabbage seeds and upon commercial seeds.*

Method [a] of treatment and strength of the solution.	Length of exposure (minutes).	Effect upon infected seeds.				Effect upon commercial seeds.									
						One-year-old seed.								Two-year-old seed.	
						Lot 3-18.[b]		Lot 4-19.[c]		Lot 5-19.[d]		Lot 2-18.[b]		Lot 2-18.[b]	
		Seed lot.	Number of seeds used.	Seeds with viable fungus after treatment (per cent).	Seeds with viable fungus and germinating (per cent).	Germination (per cent).	Seedling injury.[e]	Germination (per cent).	Seedling injury.[e]	Germination (per cent).	Seedling injury.[e]	Germination (per cent).	Seedling injury.[e]	Germination (per cent).	Seedling injury.[e]
Untreated	....	4-18	130	75.3	22.3	95-100	○	96	○	92	○	92	○	80	○
Do	....	1-19	24	66.6	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Method 1:															
Strength 1 to 1,000	10	4-18	25	56.0	4.0	99	○	.....	.....	.....	.....	.....	.....	.....	.....
	20	4-18	25	20.0	0	97	○○	.....	.....	.....	.....	.....	.....	.....	.....
	30	4-18	24	16.6	4.1	99	○○	.....	.....	.....	.....	92	○	.....	.....
	40	4-18	25	12.0	0	99	○○	.....	.....	.....	.....	.....	.....	.....	.....
	60	4-18	25	12.0	0	99	○⊖	.....	.....	.....	.....	.....	.....	.....	.....
Strength 1 to 500	10	4-18	25	20.0	12.0	99	○⊖	.....	.....	.....	.....	.....	.....	.....	.....
	20	4-18	25	36.0	8.0	98	○	.....	.....	.....	.....	.....	.....	.....	.....
	[f] 20	1-19	10	20.0	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	30	4-18	25	12.0	4.0	98	○	93	○	94	○	88	○	85	○
	[f] 30	1-19	10	50.0	20.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	40	4-18	25	12.0	4.0	98	⊖	.....	.....	.....	.....	.....	.....	.....	.....
	60	4-18	39	12.8	2.5	93	⊖	98	○	90	○	81	⊖	82	○
	60	1-19	22	63.6	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	120	4-18	24	0	0	.....	.....	85	⊖	77	⊖	63	⊖	71	⊖
	120	1-19	25	4.0	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	180	4-18	25	0	0	.....	.....	84	⊖	70	⊖	57	÷	63	÷
	180	1-19	28	28.5	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Method 2:															
Strength 1 to 500	30	4-18	13	15.3	....	87	⊖⊖	.....	.....	.....	.....	72	⊖⊖	.....	.....
	60	4-18	25	0	0	64	⊖⊖	.....	.....	.....	.....	74	⊖⊖	65	⊖
	60	1-19	25	8.0	8.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	120	....	....	....	....	.....	.....	.....	.....	.....	.....	53	⊖	.....	.....
Method 3:															
Strength 1 to 500	10	....	....	....	....	.....	.....	.....	.....	.....	.....	.....	.....	81	○
	20	....	....	....	....	.....	.....	.....	.....	.....	.....	.....	.....	78	○○
	30	1-19	24	20.8	8.3	.....	.....	.....	.....	.....	.....	.....	.....	74	○○
	60	....	....	....	....	.....	.....	.....	.....	.....	.....	.....	.....	42	+
	120	....	....	....	....	.....	.....	.....	.....	.....	.....	.....	.....	13	++
Method 4:															
Strength 1 to 500	10	....	....	....	....	.....	.....	94	○	.....	.....	.....	.....	.....	.....
	20	....	....	....	....	.....	.....	95	○○	.....	.....	.....	.....	.....	.....
	30	....	....	....	....	.....	.....	95	○○	.....	.....	.....	.....	.....	.....
	60	1-19	[g] 50	62.0	2.0	.....	.....	83	○⊖	.....	.....	.....	.....	.....	.....
	120	....	....	....	....	.....	.....	51	⊖	.....	.....	.....	.....	.....	.....

[a] Method 1=not soaked previous to treatment, rinsed after treatment; method 2=not soaked previous to treatment, not rinsed after treatment; method 3=soaked in water previous to treatment, rinsed after treatment; method 4=dipped in 70 per cent alcohol previous to treatment, rinsed after treatment.
[b] Wisconsin Hollander variety grown at Racine Wis., in 1918.
[c] All Seasons variety grown at La Conner, Wash., 1919.
[d] Jersey Wakefield variety grown at La Conner, Wash., 1919.
[e] Symbols used: ○=No injury; ⊖=slight; ÷=medium; +=severe; ++=very severe.
[f] Disinfected by seed grower.
[g] Dipped in 95 per cent instead of 70 per cent alcohol.

With the second method of treatment (soaking in the disinfectant without rinsing in water), seedling injury occurred after a shorter period, evidently as a result of the prolonged action of the disinfectant. No advantage of this over the first method was found, however, since the action upon the fungus was no more rapid than upon the host.

A limiting factor in the use of mercuric-chlorid solution is the fact that complete contact with the seed coats is usually prevented by the presence of small air bubbles. To overcome this difficulty Hutchinson and Miller (*4*) conducted their seed treatments in partial vacuum, but with only limited success. Since two trials with infected seed by this treatment in 1:500 mercuric chlorid gave no better results than parallel experiments at atmospheric pressure, the method was abandoned (see Table IV).

In order to overcome this difficulty in another way the seed was soaked in water previous to treatment (method 3). The action of the fungicide on the seeds was more rapid than with the unsoaked seed, marked injury resulting from treatments of more than 30 minutes' duration. At this point, however, the disinfection of diseased seed was no more complete than with similar exposures of unsoaked seed.

In the fourth method of treatment, where seeds were rinsed in alcohol before exposure to the mercuric-chlorid solution, the action of the disinfectant was hastened somewhat, injury to the seed being pronounced after a treatment of one hour (see lot No. 4–19, Table IV). The method appears to be no more effective than the first, however, in eradicating the fungus from infected seeds.

The results with mercuric chlorid may be summarized as follows: With most lots of seed used a soaking of one hour in a 1:500 solution followed by rinsing in water caused very slight seed injury. One lot, however, was injured considerably by this treatment. Treatment was necessary for two hours for complete eradication of the fungus in the case of one lot of infected seed, and in another lot treatment for three hours was insufficient to attain this result. Other methods used with mercuric-chlorid solution—(1) soaking in the disinfectant without rinsing after treatment, (2) presoaking in water before treatment, (3) dipping in alcohol before treatment, and (4) treatment in partial vacuum—did not appear to be superior to the first. For general use soaking for 30 minutes in a 1:1,000 solution, followed by rinsing in water, is advisable. Most lots of seed will stand a 1:500 solution for a somewhat longer time, but preliminary trials should be made before this stronger treatment is applied.

SUMMARY OF LABORATORY SEED-TREATMENT EXPERIMENTS.

The data presented justify the conclusion that no known method of cabbage-seed disinfection can be relied upon for the complete eradication of the black-leg fungus, *Phoma lingam*, without severe injury to the seed. It is also evident that different lots of seed vary widely in their relative susceptibility to injury from seed treatment. This forces one to be the more conservative in recommendations for general practice. The results of experiments lead the writer to

doubt whether dry heat can be used successfully on a commercial basis because of the wide range of susceptibility to injury in different lots of seed and because of the difficulty of application. The experiments conducted with hot water have not shown it to be superior to the chemical fungicides; so in view of the awkwardness of its application it is not at present considered suitable for general use. Further comparative trials, however, should be made. With the chemical fungicides, treatment stronger than a 1:256 or 1:240 solution of formaldehyde and a 1:1,000 solution of mercuric chlorid for 30 minutes, followed by rinsing, is unsafe for general use. Of these two treatments the mercuric chlorid seems to be slightly superior in eradicating the fungus. It is true, however, that many lots of seed will stand much more severe treatment, especially with mercuric chlorid, but in the event of such treatment preliminary tests should always be made.

FIELD TRIALS WITH TREATED SEEDS.

The results from seed treatment showed that complete elimination of seed infection is impracticable. It was evident, however, that the more effective treatments, such as 1:1,000 and 1:500 mercuric-chlorid solution and 1:256 formaldehyde solution for 30 minutes destroyed or inhibited so much of the Phoma as greatly to reduce the percentage of seedling infection. The question arose, therefore, as to whether any of the foregoing treatments would control the disease for practical purposes. In general, infected seeds were less resistant than the fungus within them. It was reasonable to expect, then, that treatment of commercial seed bearing an ordinary amount of infection would reduce the number of germinable diseased seeds to a very low percentage.

Field observations have shown that the rapidity with which the disease develops and spreads from primary centers in the seed bed varies greatly from year to year. Comparison of climatic conditions with the occurrence of the disease in different localities and in the same locality in successive years has indicated that the amount of rainfall and the atmospheric humidity are the chief factors in causing these variations. McAlpine (*6*) has pointed out that the disease in Australia thrives best in wet weather followed by heat or when the plants are forced by excessive watering. This view is further strengthened by the fact that a good supply of moisture is necessary to bring about the discharge of pycnospores of *Phoma lingam* and that they are at the same time best adapted to dissemination by spattering rain or surface drainage water.

DEVELOPMENT OF THE DISEASE IN THE SEED BED.

As described by Henderson (*3*), primary infection of germinating seedlings takes place at some point on the cotyledon or at the base

of the hypocotyl. In the first case invasion by the fungus takes place before or during germination. In rare cases the testa remains attached to one of the cotyledons, and delayed infection may thus occur. The disease appears as shriveling of the infected tissue of the cotyledon without much loss of color, beginning usually at the margin and progressing toward the petiole. Pycnidia later appear in the shrunken tissue, their development being materially hastened in humid environments. Infection at the base of the hypocotyl usually takes place after germination, inoculum coming from the fungus within the seed coat, which ordinarily remains below ground, closely adjacent to the crown. In the same way nongerminable infected seeds may be the source of inoculum for near-by healthy seedlings. This has been shown to take place by planting such seeds alternately in the furrow with healthy viable ones. In the case of hypocotyl infection, the disease appears above ground as a gradual shrinkage of the succulent tissue, progressing upward and ultimately causing a collapse of the plant. As in the case of cotyledon infection, pycnidia later appear in the shrunken tissue, their development likewise being enhanced by moist environment. Under greenhouse conditions where a temperature of 70° to 75° F. and a relative humidity of about 60 to 70 per cent were maintained, the first signs of disease usually appeared in 8 to 10 days after planting, and other primary lesions continued to develop for three to five weeks. As a rule, cotyledon lesions appeared first and hypocotyl infections a few days later. Out of doors, where there is a greater range in environmental conditions, the progress of the disease may be delayed.

RELATION OF RAINFALL TO THE DEVELOPMENT OF THE DISEASE.

A study of the effect of variation in the depth of rainfall on the development of the disease in the seed bed was made during the spring and summer of 1919 at Madison, Wis. Untreated seed of the Wisconsin Hollander variety, lot No. 2–18 previously referred to, was sown on May 14. A small percentage of the pods from which this seed was taken had shown black-leg lesions, and in greenhouse tests about 2 per cent of the seedlings had developed primary hypocotyl infections. The seed bed was divided into four plats, which were, respectively, handled as follows: Plat 1, exposed to natural weather conditions; plat 2, exposed as plat 1 and sprinkled several times each week during dry weather; plat 3, covered with a cold-frame during rains; plat 4, covered every evening and during rainy weather. The last treatment was devised to reduce the amount of dew which might form upon the plants. As a matter of fact, however, under the climatic conditions which prevailed during the trial, this artificial inclosure led to a greater accumulation of moisture upon these plants in plat 4 than upon those in plat 3. The protected

plats (Nos. 3 and 4) were watered artificially, care being taken to avoid splashing and the distribution of pycnospores.

A few infection centers appeared in all plats on or shortly after June 9. Following this date the appearance of new centers and subsequent spread was greatest in plat 2 and nearly as rapid in plat 1,

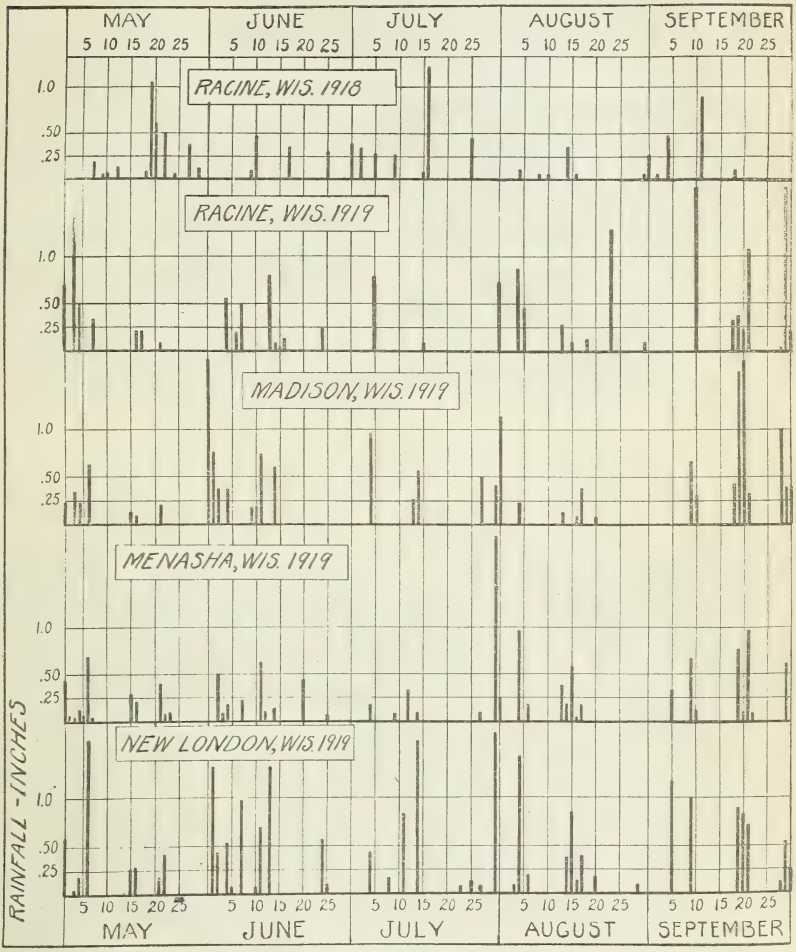


FIG. 1.—Precipitation records made by the United States Weather Bureau at Racine, Wis., in 1918 and 1919 and at Menasha, Wis. (nearest station to Appleton), and New London, Wis. (nearest station to Shiocton), in 1919.

while in the protected plats very little spread took place. The rainfall which occurred at Madison during the experiment is recorded in figure 1. The beds were kept under observation until August 4, which is about four weeks beyond the normal date for transplanting. The final estimate of the extent of the disease made at this time is presented in Table V. It is clear that where the splashing action of

rain was eliminated the spread of the disease was completely checked, while it was enhanced where artificial sprinkling was done. Although plat 3 was exposed to numerous heavy dews, these were apparently insufficient for the dissemination of spores for any appreciable distance.

TABLE V.—*Effect of variation in rainfall on the development of black-leg on cabbages in the seed bed at Madison, Wis., in 1919.*

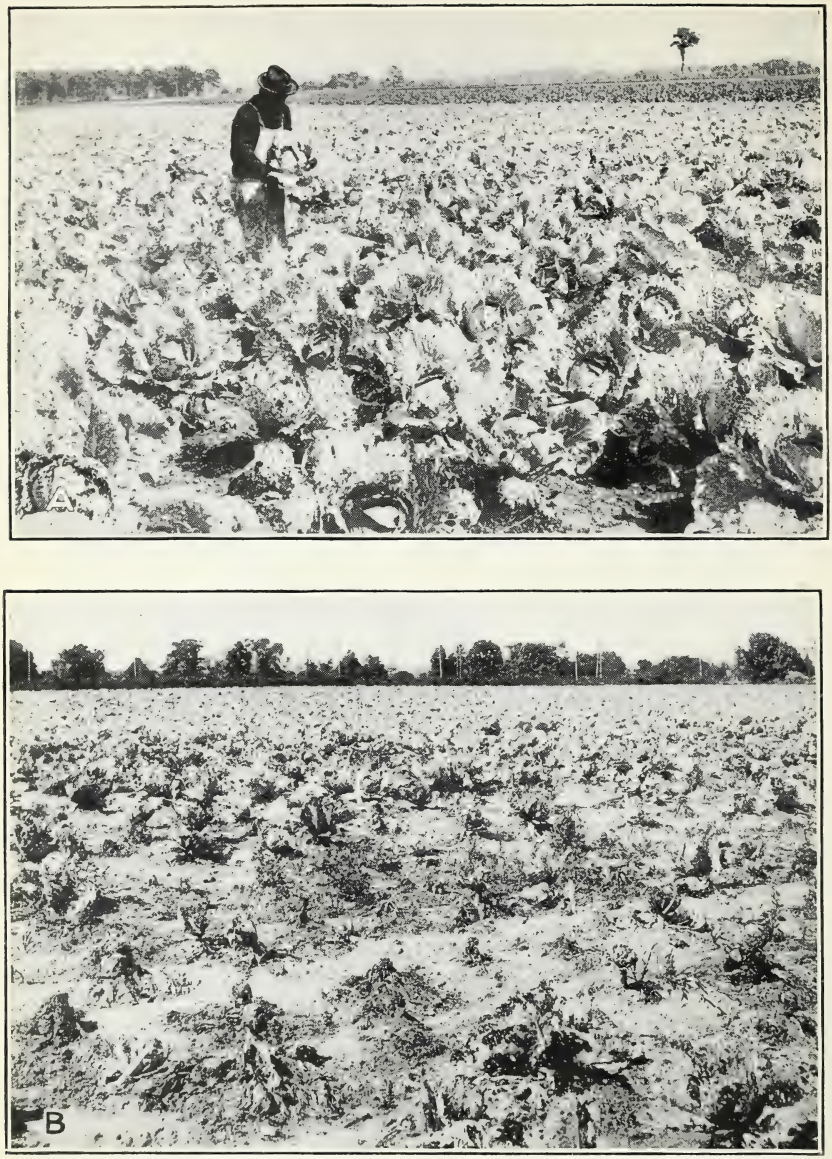
Plat.	Treatment.	Extent of disease at the end of the experiment.	
		Number of plants examined.	Diseased (per cent).
No. 1	Exposed to natural rainfall	425	28.7
No. 2	Artificially sprinkled	174	37.9
No. 3	Covered during rains	359	.5
No. 4	Covered every evening and during rains	369	2.9

This experiment was repeated in 1920. Two lots of seed, one heavily infected (No. 2–19) and one mildly infected (No. 7–19), were planted in clean soil on May 12. Part of the plat was protected from rains during the growth of the plants, water being supplied artificially, with care to avoid splashing. After the first appearance of the disease on May 22, primary infections continued to develop on the cotyledons and at the base of the hypocotyl with about equal rapidity in the protected and unprotected portions of the plat. Pycnidia in these lesions were first noted on May 25. By June 14 practically all the primarily infected plants were dead, and each bore many mature pycnidia. The first secondary infections were noted on June 17, and from that time on the disease developed rapidly on leaves and stems of the plants in the unprotected portion of the plat. In an adjoining plat healthy plants were sprayed with a suspension of spores taken directly from lesions on infected plants. The disease appeared on these plants about 15 days later. It is thus to be expected that even under favorable conditions for dissemination and infection the appearance of secondary lesions would not ordinarily take place until two or three weeks after pycnidia appeared on plants infected from the seed. The plants in the plat were large enough for transplanting by the end of June, when they were pulled and examined for the presence of the disease. The results are given in Table VI. In spite of the fact that primary infections were very numerous in the case of the No. 2–19 seed very little spread to the aerial portions of surrounding plants occurred where the splashing of rain was avoided. Likewise, with the No. 7–19 seed the extent of the dissemination was

CABBAGE BLACK-LEG.

A.—Total loss of crop due to black-leg in spite of seed treatment with a 1:256 formaldehyde solution for 30 minutes. This field is typical of the epiphytotic of black-leg which developed in the Racine, Wis., district in 1918.

B.—The importance of seed-bed infection as influencing the subsequent development of black-leg in the field. The major portion of this field was planted from a healthy seed bed, but occasional lots of plants were used from a diseased bed. The row in the center of the illustration was planted with the latter. Note that practically every plant has wilted, while there is no evidence of damage by spread of the fungus from these plants to the adjacent rows. Photographed at Racine, Wis., September 30, 1917, about 12 weeks after transplanting and shortly before harvest.



CABBAGE BLACK-LEG. RELATION OF SPREAD IN THE SEED BED TO SUBSEQUENT DEVELOPMENT IN THE FIELD.

These illustrations show the wide difference in destructiveness of the disease in plantings from the same seed bed made on different dates and in two neighboring fields. The seed was treated by the grower with 1:1,000 mercuric chlorid for 30 minutes before sowing. When the first planting was made, on June 11, practically no black-leg was noted in the seed bed, while on July 1, when the second planting was made, the disease had become widespread in the bed. Photographed at Racine, Wis., September 27, 1919, shortly before harvest.

A.—Planting of June 11. Of these plants 31 per cent were affected with black-leg, but only 4 per cent were prevented from heading by the disease. (See Table IX, field No. 1.)

B.—Planting of July 1 in the foreground. Of these plants 97 per cent were diseased and 60 per cent were prevented from heading by black-leg. The portion of the field in the background is an earlier planting from a different seed bed. (See Table IX, field No. 2.)

very small in the protected portion of the plat as compared with that in the portion exposed to natural rainfall.

TABLE VI.—*Effect of variation in rainfall on the development of black-leg on cabbages in the seed bed at Madison, Wis., in 1920.*

Seed lot.	Treatment.	Extent of disease at the end of the experiment.	
		Number of plants examined.	Diseased (per cent).
No. 2–19	Covered during rains	94	2.1
No. 2–19	Exposed to natural rainfall	117	81.1
No. 7–19	Covered during rains	135	4.4
No. 7–19	Exposed to natural rainfall	355	49.8

It is, therefore, undoubtedly true that in regions where cabbage plants are grown in open seed beds, variation in the rainfall which prevails during the period between the appearance of primary pycnidia and transplanting has a very great influence upon the development of black-leg. This fact should also be of value in checking the disease by avoiding the splashing of water where the plants are grown in covered coldframes or in greenhouses.

IMPORTANCE OF SPREAD IN THE SEED BED AS COMPARED WITH DISSEMINATION IN THE FIELD.

The question of the importance of the spread in the seed bed as compared with that in the field is, of course, to be considered in interpreting the development of the disease in midseason or later. It is natural to expect that the greatest amount of dissemination of the fungus from plant to plant takes place under the crowded condition in the seed bed and during the process of pulling and setting plants. Henderson (*3*) found that when plants showing no visible signs of the disease were taken from an infected seed bed and set into clean soil, a high percentage of them developed typical black-leg lesions on stems and roots within a few weeks. An instance is cited later in this paper (see fields 1 and 2 in Table IX; also Pl. II) where early and late planting from the same seed bed resulted in a wide difference in the destructiveness of the disease, due to the dissemination of the fungus after the first plants were removed. Numerous field observations have been made where black-leg wilt affected alternate plants in a row, as a result of the fact that one of the two droppers on the transplanting machine[3] had set diseased plants. Many cases have

[3] The machine referred to is the transplanter commonly used in many sections for setting out cabbage, tobacco, and tomato seedlings. The setting is done by two persons, who place the plants alternately in the furrow, coincident with the release of a small quantity of water from a supply tank. One row is planted at a time.

been observed where alternate rows or groups of rows in the main field, from two different seed beds, resulted in one lot of plants wilting badly from black-leg, while the other was not affected. An instance of this is shown in Plate I, *B*. Thus, although exact experimental data have not been obtained, these general field observations indicate that, under Wisconsin conditions at least, the spread of the disease in the main field is not nearly so important as that in the seed bed or during transplanting.

SEED-BED TRIALS AT MADISON, WIS., IN 1919.

In order to supplement experiments performed in the laboratory, treated samples of seed from lots Nos. 2–18 and 3–18 were sown out of doors. Each of these lots contained a small percentage of infected seed. A level strip of silt-loam soil was selected, which had not grown cabbage for at least seven years. Single-row plats 7 feet long and 18 inches apart were used. Two-gram samples of each treatment were planted on May 10 and 12. The various treatments included with the final results obtained are given in Table VII.

In the check plats of untreated seed which were sown on May 14 the disease appeared on June 9 and was fairly well advanced by July 1, the normal time for transplanting. In all the treated plats the appearance of the disease was materially checked. On June 20 four centers of disease were found, one in each of the 12, 24, and 48 hour treatments of lot No. 2–18 with dry heat at 85° C., and one in the 24-hour dry-heat treatment of lot No. 3–18 at the same temperature. In the last case the disease had spread to 15 adjoining plants and to one of the adjoining plants in the case of another center. On June 27 one infected plant each was found in the formaldehyde 1:256 30-minute and the 1:128 1-hour plats. Thus, up to that date very little disease had developed, and had the plants been set in the field at this time, which would have been the normal time for transplanting, the disease would probably have been very successfully controlled.

In order to give the parasite all possible opportunity to develop in the plats, however, the plants were not disturbed until July 31, when they were all pulled and examined for black-leg. The data given in Table VII show that the disease continued to develop and spread from primary centers during July. Very few plats were entirely free from the disease, which is in general accord with the result of laboratory experiments.

TABLE VII.—*Development of black-leg in cabbage seed-bed plats sown with 2-gram samples of treated seed at Madison, Wis., in 1919.*

Type of treatment and temperature or concentration.	Length of exposure.	Effect upon badly infected seeds—viable fungus after treatment (per cent).[a]	Effect upon slightly infected commercial seeds.					
			Lot 2-18.[b]			Lot 3-18.[b]		
			Germination (per cent).	Seedling injury.[c]	Plants infected at the end of the experiment (per cent).	Germination (per cent).	Seedling injury.[c]	Plants infected at the end of the experiment (per cent).
Untreated		75.3	92	○	29	96	○	7
Mercuric chlorid:								
Strength 1 to 1,000	30 minutes, rinsed.	16.6	92	○	16	.....	.....	.....
Strength 1 to 500	30 minutes, rinsed.	12.0	88	○	62	.....	.....	.....
	30 minutes, unrinsed.	15.3	72	⊖	22	.....	.....	.....
	1 hour, rinsed.	12.8	81	⊖	12	.....	.....	.....
Formaldehyde, not rinsed after treatment:								
Strength 1 to 256	30 minutes	.....	92	○	5	.....	.....	.....
	30 minutes	21.7	70	+	1	.....	.....	.....
Strength 1 to 128	1 hour	0	73	++	0	.....	.....	.....
	2 hours	0	46	++	2	.....	.....	.....
Hot, 1 to 128 formaldehyde:								
	1 minute	.....	45	++	4	.....	.....	.....
At 55° C.	2 minutes	.....	46	++	2	.....	.....	.....
	5 minutes	.....	28	++	1	.....	.....	.....
	1 minute	.....	32	++	1	.....	.....	.....
At 60° C.	2 minutes	.....	22	++	12	.....	.....	.....
	5 minutes	.....	20	++	0	.....	.....	.....
Hot water:								
	10 minutes	.....	83	○	0	.....	.....	.....
At 50° C.	20 minutes	.....	81	○	0	.....	.....	.....
	30 minutes	.....	86	○	1	.....	.....	.....
	1 hour	0	85	○	0	.....	.....	.....
	5 minutes	20.0	81	⊖	0	.....	.....	.....
At 55° C.	15 minutes	4.0	67	÷	3	.....	.....	.....
	30 minutes	0	52	÷	3	.....	.....	.....
	5 minutes	.....	59	÷	3	.....	.....	.....
At 60° C.	10 minutes	.....	34	+	0	.....	.....	.....
	15 minutes	.....	11	+	3	.....	.....	.....
Dry heat:								
	12 hours	32.6	88	○	19	92	○	7
	24 hours	17.7	85	○	31	98	○	24
At 85° C.	48 hours	36.0	74	÷	13	69	⊖	6
	72 hours	9.0	55	÷	.....	74	⊖	.5
	96 hours	0	53	÷	?	78	⊖	.5
	12 hours	25.0	78	÷	1	93	○	3
At 90° C.	24 hours	4.0	29	÷	1	63	⊖	2
	36 hours	4.1	30	+	0	57	+	?
	12 hours	2.8	66	÷	3	79	○	2
At 95° C.	18 hours	0	33	+	2	.....	.....	.....
	24 hours	0	9	+	3	65	⊖	0

[a] Data summarized from Tables I to IV, lots Nos. 4–18 and 1–19.
[b] Wisconsin Hollander variety, grown in 1918.
[c] Symbols used: ○=No injury; ⊖=slight; ÷=medium; +=severe; ++=very severe.

It is evident from these trials that complete disinfection of cabbage seed infected with *Phoma lingam* without material reduction in germination is not to be hoped for. It is possible that some of the stronger treatments may be used with certain lots of seed, but their recommendation for general use is out of the question. Moreover, it is unsafe to assume that treatment, even if carried to the point where reduction in germination and seedling injury begins, can

always be relied upon to yield successful control. This point will be emphasized further by data from commercial trials presented in the following pages.

RESULTS WITH TREATED SEEDS IN COMMERCIAL FIELDS.

To determine the practical value of cabbage-seed treatment, several lots of commercially grown seed, varying in weight from 2 to 400 pounds, were treated before distribution in 1918 and 1919. All the seed except lot No. 14–18 was grown in the vicinity of Racine, Wis., and most of it was kept under the writer's observation. Varying amounts of black-leg prevailed on the seed plants, but in no case was a high percentage of pods infected. As noted before, the most seriously diseased lot, No. 2–18, when tested in the greenhouse showed primary infections on approximately 2 per cent of the plants. Unless otherwise stated, the seed was disinfected by the writer. It should be noted that in the cabbage sections where these observations were made, seed is sown in open beds about the first week of May, while transplanting is done as a rule between June 15 and July 5. Where possible, a survey of the seed beds was made before transplanting was begun, and final notes were taken in representative fields at the end of the season.

RESULTS IN 1918.

All of the fields under observation in 1918 were located at Racine, Wis., and were grown from seed which had been treated with a 1:256 formaldehyde solution for 30 minutes. Seed beds were sown about April 25. When a survey of the beds was made on June 14 the disease was already quite prevalent. Most of the transplanting was done between June 15 and June 30. There was no reason to believe that infection had originated otherwise than by way of the seed, since most of the beds were on soil new to cabbage or on which the crop had not been grown for several years. The rainfall records for this section (fig. 1) show that previous to June 14 the weather had been favorable for the rapid spread of the disease from primary centers.

As a result of the early spread of the fungus in the seed bed, the disease became very destructive as the season progressed. This development was confined largely to the home-grown seed, in spite of the fact that this had all been treated. A summary of the amount of disease in five representative fields at the end of the season is given in Table VIII. A typical field grown from this seed as it appeared at the end of the season is shown in Plate I, *A*. It was clear from this season's results that treatment with a 1:256 formaldehyde solution does not successfully control the disease when sufficient rainy weather prevails to bring about the dissemination of the fungus in the seed bed.

TABLE VIII.—*Development of cabbage black-leg in commercial fields planted with treated seeds in the Racine, Wis., district in 1918.*

Seed lot.	Treatment.	Field No.	Diseased plants.		Date of observation.
			Infected.	Prevented from heading.	
			Per cent.	*Per cent.*	
No. 2–17	1:256 formaldehyde, 30 minutes, rinsed	2	87	87	Nov. 2
No. 3–17 [a]	do	3	99	96	Do.
No. 3–17 [a]	do	1	99	91	Nov. 5
No. 4–17 [a]	do	1	74	14	Do.
No. 5–17	do	4	37	52	Oct. 30
No. 5–17	do	5	83	65	Nov. 1
Average, five fields			79.8	67.5	

[a] Treated by seed grower.

RESULTS IN 1919.

The observations were continued at Racine in 1919 and were extended to the Appleton and Shiocton districts in east-central Wisconsin, where certain quantities of the No. 2–18 and No. 3–18 lots of seed had been distributed. Most of the seed in all of these sections was sown about May 9. Because of the poor showing made with the formaldehyde solution in 1918, treatment with a 1:500 solution of mercuric chlorid for 30 minutes was recommended for general use in the spring of 1919. A small quantity of untreated No. 2–18 seed was sown in the Racine district on May 9. As already noted in the experiments conducted at Madison, the disease developed first and most rapidly from the untreated seed. When the beds at Racine were examined on June 12, black-leg had become widespread in the plant ng of untreated No. 2–18 seed. From the treated seed, however, very little of the fungus had developed at this date. Most of the transplanting was done within the next two weeks, and the disease was effectively checked in most fields, as shown by the results of a survey at the end of the season (Table IX). This was undoubtedly due to the fact that the development of primary infections was retarded and the number reduced by mercuric-chlorid treatment coupled with the fact that only two rainy periods occurred during the remainder of the month—one on June 13–16 and the other on June 24. (See fig. 1.)

In one bed under observation, where practically no black-leg was noted on June 11, the disease had become widespread by July 1. Two plantings were made from this bed, the first (field 1, Table IX) about June 12 and the second (field 2, Table IX) about July 2. A comparison of the two fields at the end of the season, as given in Table IX and illustrated in Plate II, brings out the effect of extensive spread of the disease in the seed bed previous to transplanting. In

general, however, the disease was not very prevalent in Racine fields at the end of the season. Where the mercuric-chlorid and dry-heat treatments were combined, very little disease developed, but since mercuric chlorid alone controlled the disease as well in this locality, the superiority of the double treatment was not demonstrated.

TABLE IX.—*Development of cabbage black-leg in commercial fields grown from treated seeds in Wisconsin in 1919.*

Locality and seed lot.	Treatment.	Field No.	Diseased plants.		Date of observation.
			Affected.	Prevented from heading.	
Racine:			*Per cent.*	*Per cent.*	
15-18 [a]	1 to 1,000 $HgCl_2$ 30 minutes, rinsed	1	31.0	4	Sept. 19
15-18 [a]	do	2	97.0	60	Do.
13-18 [a]	1 to 500 $HgCl_2$ 30 minutes, rinsed	3	.....	11	Oct. 12
2-18 [a]	do	3	.....	3	Do.
2-18 [a]	do	4	25	25	Oct. 13
2-18 [a]	do	5	2	2	Do.
2-18 [a]	do	6	11	11	Do.
2-18 [a]	do	7	4	4	Oct. 11
3-18 [a]	do	8	.....	1	Sept. 19
3-18 [a]	do	9	2	2	Oct. 13
3-18 [a]	do	10	1	1	Do.
3-18	1 to 1,000 $HgCl_2$ 20 minutes, rinsed, plus dry heat 85° C. for 72 hours.	11	1.2	1.2	Do.
3-18	1 to 1,000 $HgCl_2$ 20 minutes, rinsed, plus dry heat 90° C. for 36 hours.	12	[b]	[b]	Do.
3-18	1 to 1,000 $HgCl_2$ 20 minutes, rinsed, plus dry heat 95° C. for 12 hours.	13	.25	.25	Do.
Average, 5 fields, lot No. 2-18.	1 to 500 $HgCl_2$ 30 minutes, rinsed	.....	10.5	9.0	.....
Average, 3 fields, lot No. 3-18.	do	.....	1.0	1.3	.....
Appleton:					
2-18	1 to 1,000 $HgCl_2$ 30 minutes, rinsed	14	100	90	Oct. 10
2-18	do	15	100	68	Do.
2-18	1 to 500 $HgCl_2$ 30 minutes, rinsed	15	92	63	Do.
Average, 3 fields, lot No. 2-18.	1 to 1,000 $HgCl_2$ 30 minutes, rinsed	.....	.....	.....	.....
	1 to 500 $HgCl_2$ 30 minutes, rinsed	.....	97.3	73.6	.....
Shiocton:					
3-18 [a]	do	16	50 to 90	50 to 90	Oct. 11
3-18 [a]	do	17	50 to 90	50 to 90	Do.
3-18 [a]	do	18	50 to 90	50 to 90	Do.
14-18 [a]	1 to 256 HCHO 30 minutes, rinsed	19	.....	90	Do.
Average, 3 fields, lot No. 3-18.	1 to 500 $HgCl_2$ 30 minutes, rinsed	.....	50 to 90	50 to 90	.....

[a] Disinfected by seed grower. [b] One diseased plant found in a 3-acre field.

Conditions were decidedly different in the Appleton and Shiocton districts. A survey showed that the disease had developed into a uniformly severe epiphytotic in fields of both localities grown from Nos. 2–18 and 3–18 seed (Table IX). Unfortunately the seed beds were not examined, but the even distribution and advanced stage of the disease left no doubt that the fungus had become widespread before transplanting. In one field at Shiocton where the formaldehyde treatment was used the disease was not controlled. A comparison of precipitation records (fig. 1) taken at Menasha (near

Appleton) and at New London (near Shiocton) with those taken at Racine shows that the rainy periods from May 15 to June 15 were more numerous and as a rule of longer duration in the first two localities. It may be inferred from this that there was more opportunity for pycnospore dissemination and subsequent infection at Appleton and Shiocton than at Racine. In view of the evidence already presented as to the importance of rainfall in this connection, it is not improbable that this factor was the critical one in determining the difference in development of the disease in the three localities mentioned.

These field trials justify the conclusion, as did those made in the laboratory, that neither formaldehyde nor mercuric-chlorid treatment of cabbage seed, even if carried to the point where seed injury occurs, is a sure preventive of black-leg. Such seed treatment does, however, greatly reduce the development of primary infection. This result, if coupled with favorably dry weather during the seed-bed period, may suffice to give practical disease control, but with more abundant rains during this period the disease may develop to a serious extent. It should be recalled in this connection that seed treatment with corrosive sublimate is also a successful preventive of seed-borne black-rot organisms (*12*). Since cabbage-seed treatment is a useful precaution in these two possible directions and is so simple and inexpensive, it is strongly recommended. In the light of our present knowledge, soaking in a 1:1,000 mercuric-chlorid solution for 30 minutes followed by rinsing in clean water is safest for general use.

IMPORTANCE OF DISEASE-FREE SEEDS.

The fact has been brought out that in spite of any practicable seed treatment abundant rainfall may cause sufficient spread of the black-leg fungus from the few primary infections appearing in the seed bed to produce an epiphytotic. Since, therefore, in practice one can not rely with confidence upon cabbage-seed treatment for the sure elimination of the black-leg organism from infected seed, it is obviously important to give increased attention to the securing of seed free from Phoma infection. Experience in Wisconsin has shown that to accomplish this the crop must be kept clean from the outset; in other words, disease-free "mother seed" must be used on clean soil. A large percentage of American cabbage seed is now grown in two sections, eastern Long Island, N. Y., and in the Puget Sound region of western Washington. In the latter section "mother seed" is usually obtained from another locality, most commonly from Long Island, Denmark, or England. In the Long Island section local or foreign grown "mother seed" is used. In either case the danger of introducing black-leg into the seed crop through infected seed is very great, since practically none of the growers practice seed treatment.

This was brought very forcibly to the attention of the writer in August, 1920, when such a case was described by a seed contractor in one of the mentioned localities. Stock "mother seed" of a given variety which later was proved to be infected with Phoma was distributed among several seed growers in 1919. A trial row was planted in 1920 by the contractor, and when examined in August by the writer the plants showed a high percentage of black-leg. One of the 1919 plantings for seed examined in 1920 showed black-leg infection on 25 per cent of the seed plants,[4] while the several other fields from the same "mother seed" were undoubtedly infected. Thus, approximately 2,000 pounds of commercial seed became infected as a result of the introduction of the parasite on a small quantity of stock seed. Much of such seed infection can be prevented by growing stock seed from seed heads carefully selected for freedom from disease followed by careful inspection during the following season. By applying this method in Wisconsin black-leg has been successfully avoided and comparatively large quantities of disease-free "mother seed" have been produced.

Black-leg has not as yet been reported from the Puget Sound region, and an inspection of more than 30 seed fields in that region in June, 1919, yielded no sign of the disease. Inasmuch as most of these seed fields are started from "mother seed" grown in the East or abroad and none of the growers practice seed treatment, it is inevitable that some infected seed is sown. It would seem from comparison with our Wisconsin experience that this absence of the disease, as noted in the seed fields, must therefore be attributed to local climatic conditions restrictive to the development of the fungus. This was rendered the more probable by the results previously recorded (*11*) with some Wisconsin-grown seed known to be infected with Phoma. This was seed No. 2–18, which, as used in Wisconsin, resulted in high percentages of infection before the end of the season (see Tables VII and IX). Of this, 2 pounds were sent to the Puget Sound section for propagation after treatment for 30 minutes with mercuric chlorid. This was sown at Mount Vernon, Wash., about May 25, and when it was inspected seven weeks later, July 14, only four infected seedlings were found in the entire bed. These were evidently cases of primary infection with no spread whatever to the adjacent plants. In 1920, samples from two lots of badly infected seed were sown in this locality and portions of the same lots sown at Madison, Wis. In the latter region the fungus appeared in numerous primary infections within two weeks, and the disease had become widespread by transplanting time (see Table VI). Since

[4] The writer is indebted to Mr. I. H. Vogel, of Cornell University, for information regarding this field.

personal inspection by the writer of the western plantings was impossible, notes were taken by a careful grower, aided by preserved specimens of the disease forwarded to him. Although the plantings were kept under observation until October, he noted no development of the disease, thus apparently confirming the observations of the previous season.[5] It is possible that the dry weather which usually prevails in western Washington from about June 10 to September 1 (*5*) may account for the lack of development of black-leg. This matter should, therefore, be kept under observation for a period of years, since it is obvious that relatively minor variations in amount and date of rainfall from season to season may materially affect the development of the disease. In any case, this experience emphasizes the necessity of greater attention than has heretofore been given to the importance and practicability of securing cabbage seed free from black-leg infection.

SUMMARY.

(1) Cabbage black-leg is a disease of increasing economic importance, and its appearance in epiphytotic form has been generally associated with the use of infected seed.

(2) Experimental treatments of infected and commercial seeds with formaldehyde solution, mercuric-chlorid solution, hot water, and dry heat have shown that the fungus can not be entirely eradicated from infected seeds by any of these fungicides without materially reducing germination and causing injury to seedlings.

(3) When infected seed is used primary lesions appear as a rule on a small percentage of seedlings in the seed bed 10 days to several weeks after planting.

(4) The subsequent dissemination and development of the fungus are favored by spattering water and atmospheric humidity. Consequently, where the seed bed is located in the open, spread from such primary centers to surrounding plants is dependent upon the amount of rainfall and of humid weather which prevails during the seedling stage.

(5) The dissemination of the fungus in the seed bed or during transplanting appears to be of much greater importance than its spread in the main field.

(6) Seed-bed trials showed that the disease was checked by seed treatment, but was not completely controlled even when the treatment was carried beyond the point where seed injury resulted.

(7) In 1918 an epiphytotic of black-leg developed at Racine, Wis., from seed treated with a 1:256 formaldehyde solution for 30 min-

[5] Comparative trials in Wisconsin, at Mount Vernon and Madison, were continued in 1921 with confirming results. (See Walker, J. C., and Tisdale, W. B. Further notes on the occurrence of cabbage black leg Abstracts of papers presented at the Toronto meeting of the American Phytopathological Society, Dec., 1921. *In* Phytopathology, v. 12.)

utes. This occurred under weather conditions favorable to the disease, owing to frequent rains.

(8) In 1919, in the same locality, under dry weather conditions, the disease was quite effectively controlled by seed treatment, soaking in 1:500 mercuric chlorid for 30 minutes, followed by rinsing in clean water. At Shiocton and Appleton, Wis., where longer and more frequent rainy periods prevailed between the appearance of primary centers and transplanting, serious epiphytotics developed from the same lots of seed as used in the Racine district. It was thus shown that even this strong treatment was insufficient to control the disease effectively when climatic conditions favored the rapid spread of the fungus from primary centers.

(9) Since treatment reduces the number of primary centers and retards their development somewhat and since it is also a preventive against seed-borne black-rot organisms, it should be recommended. The limitations to success in the control of black-leg, however, should be recognized.

(10) Trials have developed the fact that different lots of cabbage seed may vary considerably in their relative susceptibility to injury from seed-disinfection treatments. Stronger treatment than a 1:256 or 1:240 solution of formaldehyde or a 1:1,000 solution of mercuric chlorid for 30 minutes followed by rinsing is unsafe for general use. Of these two treatments, the mercuric chlorid seems to be slightly superior in eradicating the fungus. It is true, however, that many lots of seed will stand much more severe treatment, especially with mercuric chlorid, but in the event of such treatment preliminary tests should always be made.

(11) In view of the limitations of treatment in controlling black-leg special attention should be given to securing disease-free seed.

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